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DECLARATION OF ACCURACY OF TRANSLATION
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The undersigned traslator, having an office at

c/o Patent Department, Sankyo Co., Ltd., No. 2-58, 1-chome, Hiromachi, Shinagawa-ku, Tokyo, Japan

certifies and declares that:

(1) I am fully conversant both with the Japanese and English languages.

(2) (A) I have translated into English Japanese Patent Application Number filed . A copy of said English translation is attached hereto.

(2) (B) I have carefully compared the attached English-language translation of Japanese Patent Application Number 136449/1980 filed September 30, 1980 , with the original Japanese-language patent application.

(3) The translation is, to the best of my knowledge, and belief, and accurate translation from the original into the English language.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the matter with which this translation is used.

Date: February 18, 1983

Akio Ohno

[English Translation of Certified Copy]

PATENT OFFICE

JAPANESE GOVERNMENT

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application : September 30, 1980

Application Number : Patent Application No. 136449/1980

Applicant : Sankyo Company, Limited

Ex 6 p 38
ethoxycarbonyloxyethyl

September 3, 1981

Director-General, HARUKI SHIMADA

Patent Office

Official Seal

Certificate Serial No. 25294

Application for Patent (Patent Application pursuant to a proviso of Article 38 in Patent Law)

September 30, 1980

(5,400 yen)

To : Haruki Shimada, Director-General of the Patent Office

1. Title of Invention :

Cephalosporin compounds for oral administration

2. Number of Inventions described in claim : 5

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6. List of appended documents

(1) Specification 1 copy

(2) Drawings None

(3) Power of Attorney 1 copy

(4) Duplicate of Application 1 copy

7. Other Inventor, Patent Applicant or Attorney in addition to the foregoing person

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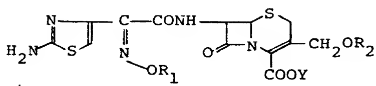
S P E C I F I C A T I O N

1. Title of the Invention

Cephalosporin compounds for oral administration

2. Scope of Patent Claim

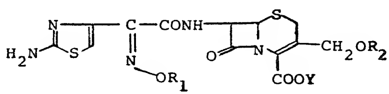
(1) A cephalosporin compound (syn isomer) having the general formula



[wherein R_1 represents hydrogen atom or a lower alkyl group, R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{\displaystyle R_3}{\underset{|}{C}}HOCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

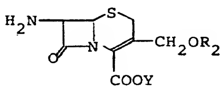
and the pharmacologically acceptable salt thereof.

(2) A process for preparing a cephalosporin compound (syn isomer) having the general formula



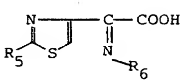
[wherein R_1 represents hydrogen atom or a lower alkyl group, R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{R_3}{\underset{|}{C}}HOCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

and the pharmacologically acceptable salt thereof which comprises reacting a compound having the general formula



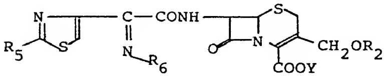
(wherein R_2 and Y are as defined above)

with a carboxylic acid having the general formula



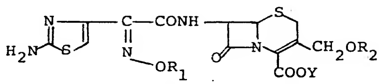
(wherein R_5 represents amino group or a protected amino group and R_6 represents hydroxyl group, a protected hydroxy group or a lower alkoxy group)

or its reactive derivative to give a compound having the general formula



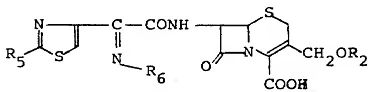
(wherein R_2, R_5, R_6 and Y are as defined above)
and, when R_5 is a protected amino group and R_6 is a protected hydroxy group, removing the protecting groups from the resulting compound.

(3) A process for preparing a cephalosporin compound (<u>syn</u> isomer) having the general formula



[wherein R_1 represents hydrogen atom or a lower alkyl group, R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{\displaystyle R_3}{\underset{|}{C}H}OCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

and the pharmacologically acceptable salt thereof which comprises reacting a carboxylic acid compound having the general formula



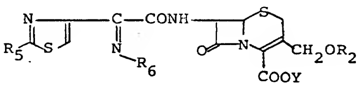
(wherein R_2 is as defined above, R_5 represents amino group or a protected amino group and R_6 represents hydroxy group, a protected hydroxy group or a lower alkoxy group)

or its reactive derivative with a phthalidyl halide or a compound having the general formula

$$\underset{\substack{|\\ R_3}}{\mathrm{HOCH}}\mathrm{OCOR_4}$$

(wherein R_3 and R_4 are as defined above)

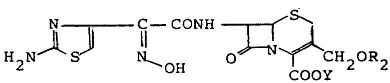
or its reactive derivative to give a compound having the general formula



(wherein R_2, R_5, R_6 and Y are as defined above)

and, when R_5 is a protected amino group and R_6 is a protected hydroxy group, removing the protecting group from the resulting compound.

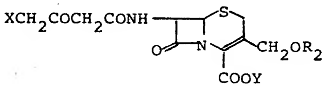
(4) A process for preparing a cephalosporin compound (syn isomer) having the general formula



(wherein R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{\substack{|\\ R_3}}{\mathrm{CH}}\mathrm{OCOR_4}$ (wherein R_3 represents hydrogen atom or methyl

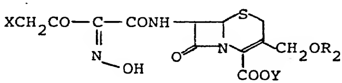
group and R_4 represents a lower alkyl group or a lower alkoxy group)

or the pharmacologically acceptable salt thereof which comprises nitrosoating a compound having the general formula



(wherein R_2 and Y are as defined above and X represents a halogen atom)

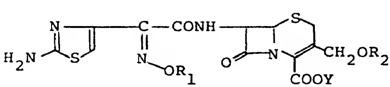
to give a hydroxyimino compound having the general formula



(wherein R_2, Y and X are as defined above)

and reacting the latter compound with thiourea.

(5) An oral treating agent for infectious disease comprising a cephalosporin compound (syn isomer) having the general formula



[wherein R_1 represents hydrogen atom or a lower alkyl group, R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula

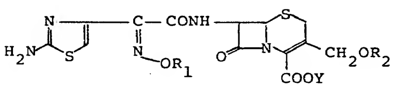
$-\underset{\substack{| \\ R_3}}{CH}OCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

or the pharmacologically acceptable salt thereof as the active ingredient.

3. Detailed Description of the Invention

This invention relates to cephalosporin compounds for oral administration.

More particularly, the present invention concerns with cephalosporin compounds (syn isomer) having the general formula



(I)

[wherein R_1 represents hydrogen atom or a lower alkyl group, R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{\substack{| \\ R_3}}{CH}OCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

and the pharmacologically acceptable salts thereof, processes for preparing said compounds and oral treating agents for

infectious diseases comprising said compounds as the active ingredient. In the above formula (I), R_1 is preferably hydrogen atom or a straight or branched alkyl group having from 1 to 4 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl, R_2 is preferably a straight or branched alkyl group having from 1 to 4 carbon aoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl and Y is preferably phthalidyl group or a group of the formula $-\underset{\underset{R_3}{|}}{C}HOCOR_4$ (wherein R_3 is hydrogen atom or methyl group and R_4 is a straight or branched alkyl group having from 1 to 4 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl or tert-butyl or a straight or branched alkoxy group having from 1 to 4 carbon atoms such as methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, sec-butoxy or tert-butoxy).

The present compounds having the above formula (I) are novel compounds which are readily absorbed through the digestive tract and converted in vivo to a carboxylic acid type compounds by elimination of the ester moiety at the 4-position. Thus, it is possible to obtain a high concentration of the carboxylic acid type compound in blood and to achieve a highly remarkable effect in treatment of infectious diseases caused by gram-positive and gram-nagative bacteria when administered orally. The carboxylic acid type compounds

are also novel and have excellent antibacterial activities as described later.

Although there are many penicillin and cephalosporin type antibiotics which exhibit excellent effects, very rarely seen are the compounds which are absorbed efficiently through the digestive tract. For this reason, there are a number of compounds of which development has been given up. Among the antibiotics of cephalosporin type, only compounds having a limited structure such as cephalexin or its analogs are offered for practical purposes.

The recovery ratio in urine of cephalosporin preparations widely used for injections such as cephalothin, cefazolin, cefmethazole when administered orally is about 5% of the dose administered, and they are known for their extremely poor absorption through the digestive tract. The reason for this is because dissociation of carboxylic group at 4-position of cephalosporin is great (pKa value is small) and the acidity is strong.

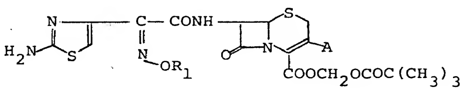
Efforts have been made to improve such absorption through the digestive tract by esterifying carboxylic groups at 3-position of penicillins or at 4-position of cephalosporins. There are one or two cases where such efforts have matured into commercialization in respect of penicillin compounds. But so for none succeeded for all efforts in the case of cephalosporin compounds.

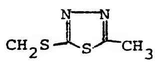
There is a report in the Journal of Antibiotics, Vol. 32, No. 11, p. 1155 (1979) regarding acetoxymethyl ester of cefamandol. According to this report, the absorption is not improved because the esterification makes the compound only slightly soluble in water, and the absorption is improved to a certain degree only when it is administered in the solution of as organic solvent such as propylene glycol. On the other hand, there is a report on an ester which is easily soluble in water in the Journal of Medicinal Chemistry, Vol. 22, p. 657 (1979) which further describes that absorption was not improved because the compound was chemically unstable.

The inventors of the present invention have conducted extensive research for many years on the improvement of absorption of cephalosporin compounds through digestive tracts by chemical modifications and on elevation of concentration in blood through oral administrations. The inventor has learned that these properties are related to the whole structure of the compounds, and the absorption through the digestive tract is utterly unpredictable even when the similar chemical modification such as esterification is conducted so long as a part of the structure is different. The present invention was achieved based on the result of such researches.

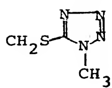
The inventor synthesized pivaloyloxymethyl esters of the known compound identified below which have seemingly similar

structure as the compounds of the present invention, and conducted experiments to study the recovery ratio thereof in through oral administration. The results obtained were not necessarily good, and the inventor found that the substituent at 3-position plays an extremely significant role as far as the compounds of this group are concerned.



Compound 1 R_1 = H A = 

" 2 $R_1 = CH_3$ A = CH_2OCOCH_3

" 3 $R_1 = CH_3$ A = 

The present compounds having the formula (I) can be prepared by the following methods:

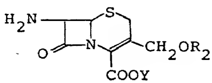
(a) Acylation of the esterified 7-amino-3-alkoxymethyl-cephalosporin (II).

(b) Esterification of the carboxylic acid (I') corresponding to the compound (I) or its amino and hydroxy-protected derivative (V) and elimination of the protecting groups.

(c) Conversion of the 7-acyl group to the desired acyl group by chemical reaction.

The above methods will more concretely be illustrated below.

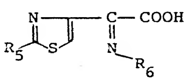
(a) The compound (I) is prepared by reacting a compound having the general formula



(II)

[wherein R_2 represents a lower alkyl group and Y represents phthalidyl group or a group of the formula $-\underset{\displaystyle R_3}{\underset{|}{C}}HOCOR_4$ (wherein R_3 represents hydrogen atom or methyl group and R_4 represents a lower alkyl group or a lower alkoxy group)]

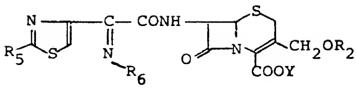
with a carboxylic acid having the general formula



(III)

(wherein R_5 represents amino group or a protected amino group and R_6 represents hydroxy group, a protected hydroxy group or a lower alkoxy group)

or its reactive derivative to give a compound having the general formula



(IV)

(wherein R_2, R_5, R_6 and Y are as defined above) and, when R_5 is the protected amino group and R_6 is the protected hydroxy group, removing the protecting groups.

In the above formulae, preferred amino-protecting groups in R_5 are those which are readily removed to restore amino group, for example, trityl group, formyl group, t-butoxycarbonyl group, or 2-ethoxycarbonyl-1-methylvinyl group, which are removable by acid treatment, 2,2,2-trichloroethoxycarbonyl group, which is removable by reduction, 2-methylsulfonylethyloxycarbonyl group, which is removable by alkali treatment, and chloracetyl group, which is removable by treatment with thiourea.

Preferred hydroxy-protecting group in R_6 are those which are readily removed to restore hydroxy group, for example, trityl group and dichloroacetyl group, which are removable by acid treatment.

The present process consists of an acylation step and, if necessary, a removing step of the protecting group.

In the acylation step, the compound having the formula (III) is used in a free form or in a form of the reactive derivative. When it is used as such, a suitable condensing agent is employed. Examples of such condensing agent include

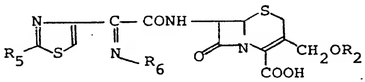
a di-substitued carbodiimide such as dicyclohexylcarbodiimide, an imidazolide such as carbonyldiimidazole or thionyldiimidazole, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, or a Vilsmeier reagent preparing from dimethylformamide and, e.g., phosphorus oxychloride or thionyl chloride. Examples of the reactive derivative of the compound having the formula (III) include the acid halide, the acid anhydride, the mixed acid anhydride, the reactive ester, the reactive amide, the acid azide, etc. Preferred mixed acid anhydrides include the mixed acid anhydride with carbonic acid mono lower alkyl ester such as monomethyl carbonate or monoisobutyl carbonate or the mixed acid anhydride with a lower alkanoic acid such as pivalic acid or trichloroacetic acid. Preferred reactive esters include p-nitrophenyl ester, pentachlorophenyl ester, N-hydroxyphthalimide ester, etc.

The present step is preferably conducted in a solvent. The solvent is not limited as far as it does not obstruct the reaction and exemplified by an inert organic solvent such as acetone, methyl ethyl ketone, tetrahydrofuran, dioxane, ethyl acetate, chloroform, dichloromethane, acetonitrile dimethylformamide, dimethyl sulfoxide, etc. or a mixture of said solvent and water. Depending on a kind of the reactive derivative employed, a base is used, if necessary. Examples of the base include an alkali metal compound, for example, sodium bicarbonate, potassium bicarbonate, sodium

carbonate, potassium carbonate, etc. and an aliphatic, aromatic or nitrogen-containing heterocyclic base, for example, triethylamine, dimethylaniline, N-methylpiperidine, N-methylpyrrolidine, pyridine, collidine, lutidine, etc. The reaction temperature is not limited and usually the reaction is conducted at room temperature or under cooling. The reaction period may be varied depending on mainly acylation methods and reaction temperature, but usually from tens of minutes to tens of hours. After completion of the reaction, the compound having the formula (IV) may be recovered from the reaction mixture by conventional means. For example, in case where water-miscible solvent is employed, the solvent is removed by distillation under reduced pressure and the residue is dissolved in a water-immiscible solvent. The resulting solution is washed with an acid and base and dried and the solvent is distilled off to obtain the product. In case where water-immiscible solvent is employed, the reaction mixture is directly washed with an acid or base. The product thus obtained, if necessary, is further purified by conventional means, for example, chromatographic techniques.

In the removing step of the protecting group, the protecting group may be removed, as mentioned above, by conventional means according to the properties thereof and the resulting crude product is purified to give the desired compound having the formula (I).

(b) Alternatively, the compound (I) can be prepared by reacting the carboxylic acid compound having the general formula



(V)

(wherein R_2, R_5 and R_6 are as defined above) or its reactive derivative with a phthalidyl halide or a compound having the general formula

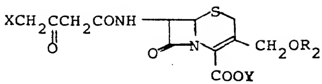
$$\underset{\displaystyle R_3}{\underset{|}{\text{HOCH}}}\text{OCOR}_4 \qquad \text{(VI)}$$

(wherein R_3 and R_4 are as defined above) or its reactive derivative to give the compound having the above formula (IV) and, when R_5 and R_6 are protected groups, removing the protecting groups from the resulting compound as mentioned in the method (a).

In the above reaction, both starting materials may be used in a free form, but it is preferable to use either or both of them in a reactive derivative form. Examples of the reactive derivatives of the carboxyl moiety in the compound (V) include the salt with a metal such as sodium or potassium, the salt with an organic amine such as triethylamine, the acid halide such as acid chloride or acid bromide, the acid anhydride, the mixed acid anhydride with a carbonate

such as ethyl carbonate or isobutyl carbonate, etc. Examples of the reactive derivatives of the alcohol group in the compound (VI) include a sulfonyl ester such as methanesulfonate or p-toluenesulfonate, a halogen-substituted derivative in which the hydroxy group is substituted with chlorine or bromine. The reaction is desirably conducted in the presence of a suitable solvent which does not obstruct the reaction. Examples of such solvent include dimethylformamide, dimethylacetamide, dimethyl sulfoxide, hexamethyltriamidophosphate, acetonitrile, etc or the mixture with other inert organic solvent. The reaction is usually conducted at room temperature or under cooling. The reaction period is usually from several minutes to several hours. After completion of the reaction, the reaction mixture is diluted with a water-immiscible solvent, washed successively with an aqueous potassium bisulfate solution and an aqueous basic solution and dried and the solvent is distilled off to obtain the desired product. The product thus obtained may be further purified by conventional means, for example, chromatographic techniques. In case where amino and hydroxy groups are protected, the groups are converted to free amino and hydroxy groups, respectively, following the procedure applied in the above compound (IV).

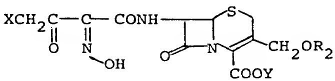
(c) The compound having the above formula (I) wherein R_1 is hydrogen atom can be prepared by nitrosoating the compound having the general formula



(VII)

(wherein X represents a halogen atom such as chlorine or bromine and R_2 and Y are as defined above)

to give the hydroxyimino compound having the general formula



(VIII)

(wherein X, R_2 and Y are as defined above)

and reacting the latter compound with thiourea.

The present process consists two steps, i.e., the nitrosoating step and the thiazole ring-forming step.

The nitrosoating step may be carried out according to conventional nitrosoation of β-diketones. Namely, it is carried out by reacting, under acidic conditions, said compound (VII) with a nitrous acid compound, for example, nitrites such as sodium nitrite or potassium nitrite, and esters of nitrous acid such as amyl nitrite or butyl nitrite. The reaction temperature is not limited and usually the reaction is conducted at room temperature or under cooling. The reaction period may be varied depending mainly on a kind of the nitrosoating agent, but usually from tens of minutes to several hours. After completion of the reaction, the compound

having the above formula (VIII) may be recovered from the reaction mixture by conventional means. For example, the desired product may be obtained by distilling off the solvent from the reaction mixture under reduced pressure. The product thus obtained may be purified by conventional means, for example, chromatographic techniques.

The thiazole ring-forming step comprises reacting an α-haloketo compound with thiourea and may be conducted by contacting the both reagents in the presence of a suitable solvent. The solvent is not limited as far as it does not obstruct the reaction. Preferred solvents include dimethylformamide, dimethylacetamide and acetonitrile, which readily dissolve the reagents. It is preferred for completion of the reaction to carry out the reaction in the presence of a base, preferably sodium bicarbonate, potassium bicarbonate, or the like. The reaction temperature is not critical. The reaction proceeds usually at room temperature. The reaction period depends on the reaction conditions, but usually is from several tens minutes to several hours. After completion of the reaction, the desired compound having the above formula (I) wherein R_1 is hydrogen atom may be recovered from the reaction mixture by conventional means. For example, after completion of the reaction, the reaction mixture is concentrated under reduced pressure. The residue is dissolved in a suitable organic solvent, washed with water and dried and the solvent is

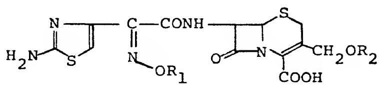
distilled off to obtain the desired product, which may be purified by conventional means, for example, chromatographic techniques.

The compounds (I) thus obtained are mentioned above, readily absorbed through the digestive tract and gives a high concentration of the carboxylic acid type compound in blood and, accordingly, oral administration of the compound is possible. The compound can be formulated with conventional pharmaceutical vehicles to form such oral dosage units as capsules, powders, granules, tablets. The pharmaceutical vehicles include diluents, for example, starch, lactose, sugar, calcium carbonate, calcium phosphate, polyethylene glycol etc., binding agents, for example, acacia, carboxymethyl cellulose, hydroxypropyl cellulose, etc., lubricants, for example, magnesium stearate, talc, etc. and disintegrants, for example, carboxymethyl cellulose calciumsalt, etc. The dose will vary depending upon age, body weight and condition at patient. From about 0.2 g. to 5 g., preferably from 0.5 g. to 3 g. of the compound can be administered daily to the human adult in 3 or 4 devided doses.

The compounds having the formula (I) can be used, as well as in a form of a free base, in a form of pharmacologically acceptable acid addition salts, for example, the salt with an inorganic acid such as hydrochloric acid, sulfuric acid or phosphoric acid, or the salt with an organic acid

such as methanesulfonic acid, benzenesulfonic acid, malonic acid, etc.

The present compounds (I), as mentioned above, are readily absorbed through the intestine by oral administration and hydrolyzed *in vivo* to produce the corresponding carboxylic acid (I') or the salt thereof.



(I')

(wherein R_1 and R_2 are as defined above)

Antibacterial activities of the compound (I') against gram-positive and gram-negative bacteria (minimum inhibitory concentrations, μg/mℓ) are extraordinarily potent as shown below.

	Compound (I')	
	A	B
	R_1=H, R_2=CH_3	R_1=R_2=CH_3
Staphylococcus aureus 209 P	0.1	0.4
Escherichia coli NIHJ	0.4	0.4
Shigella flexneri	0.8	0.8
Klebsiella pneumoniae	0.2	0.1
Proteus vulgaris	0.2	0.01
Salmonella enteritidis	0.4	0.2

The present compounds (I) and the related compounds (compound 1, 2 and 3) were applied orally to mice and their recovery rates in urine i.e. the amounts of the corresponding carboxylic acids are given below.

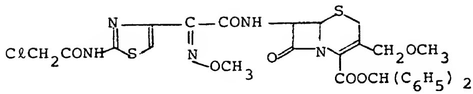
	Recovery rate in urine (%)
Compound in Example 1	above 40
Compound in Example 2	above 50
Compound 1	15
Compound 2	8
Compound 3	14

As shown above, the present invention is characterized by improvement of the absorbability of cephalosporins through digestive tracts converting the substituent at the 3-position to alkoxymethyl group.

The processes for preparing the present compounds (I) are more concretely illustrated by the following Referential Examples and Examples, which are not to be construed as limitaing the scope of this invention. All of the present compounds are syn form concerning their oxime moiety.

Referential Example 1

Preparation of diphenylmethyl 7-[2-(2-chloroacetamidothiazole-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate



(A) To 0.057 mℓ. of dimethylformamide was added 0.061 mℓ. of phosphorus oxychloride under ice-cooling and stirring. The mixture was stirred at 40 °C for an hour and subjected twice to azeotropy with dry methylene chloride. To the resulting mixture was added 1 mℓ. of ethyl acetate. Under vigorously stirring at room temperature, 200 mg. of 2-(2-chloroacetamidothiazole-4-yl)-2-methoxyiminoacetic acid was added to the above mixture and stirring was continued for 30 minutes.

(B) On the other hand, 200 mg. of diphenylmethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate and 145 mg. of diethylaniline were dissolved in 5 mℓ. of methylene chloride and stirred at -5 °C.

To the resulting mixture (B) was added dropwise the above reaction mixture (A). After stirring for 15 minutes, the reaction mixture was concentrated under reduced pressure. To the residue were added 20 mℓ. of ethyl acetate and 5 mℓ. of water. The ethyl acetate layer was separated, washed successively with 5 mℓ. of a saturated aqueous sodium bicarbonate solution, 5 mℓ. of a 5% hydrochloric acid and 5 mℓ. of a saturated aqueous sodium chloride

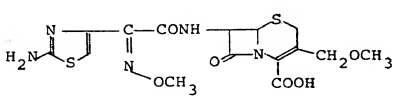
solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed through 30 g. of silica gel (Kieselgel-60) eluted with a mixed solvent of n-hexane and ethyl acetate (3 : 2) to give 213 mg. of diphenylmethyl 7-[2-(2-chloroacetylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCl_3$) δ ppm

3.19 (3H, s, OCH_3 at 3-position)
3.51 (2H, s, CH_2 at 2-position)
4.09 (3H, s, OCH_3)
4.20 (2H, s, CH_2 at 3-position)
4.22 (2H, s, $ClCH_2 \cdot CO$)
5.02 (1H, d, J = 2.5 Hz, 6-position)
5.86 (1H, d . d, J = 2.5, 4.5 Hz, 7-position)
6.7 - 7.6 (12H, m)

Referential Example 2

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-methoxyimino-acetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid trifluoroacetic acid salt



· CF_3COOH

In 5 mℓ. of N,N-dimethylacetamide were successively dissolved 200 mg. of diphenylmethyl 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyimonoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate and 45 mg. of thiourea. The solution was maintained at room temperature for 2 hours. After addition of a saturated aqueous sodium bicarbonate solution, the reaction mixture was extracted with 20 mℓ. of ethyl acetate. The ethyl acetate layer was washed with water to remove the excess thiourea and dried over anhydrous magnesium sulfate. After the drying agent was filtered off, the filtrate was concentrated under reduced pressure. The resulting residue was chromatographed through 30 g. of silica gel (Wacogel C-100) eluted with ethyl acetate to afford 63 mg. of diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 2 mℓ. of anisole and 1 mℓ. of trifluoroacetic acid was added thereto under ice-cooling and stirring. The mixture was maintained at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressure and isopropyl ether was added thereto. Produced precipitates were collected on a filter and dried to afford 27 mg. of 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid trifluoroacetic acid salt.

NMR (in deuteroacetone, heavy water was added): δ ppm

3.29 (3H, s, $-OCH_3$ at 3-position)

3.57 (2H, s, CH_2 at 2-position)

3.96 (3H, s, OCH_3)

4.27 (2H, s, CH_2 at 3-position)

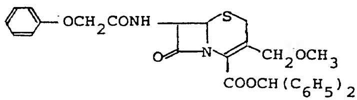
5.15 (1H, d, J = 2.5 Hz, 6-position)

5.97 (1H, d, J = 2.5 Hz, 7-position)

6.59 (1H, s)

Referential Example 3

Preparation of diphenylmethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate



In 200 mℓ. of water were dissolved 10.9 g. of 7-phenoxyacetamido-3-acetoxymethyl-3-cephem-4-carboxylic acid and 2.25 g. of sodium bicarbonate. To the solution was added 10 g. of lyophilized microorganisms of Bacillus subtillis ATCC 6633. The mixture was adjusted to pH 7.5 - 8 and stirred at 40 °C for a day. The microorganisms were filtered off. The filtrate was adjusted to pH 2 - 3 with hydrochloric acid and extracted three times with 200 mℓ. of ethyl acetate each. The extract was washed with 50 mℓ. of a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and 6.3 g. of diphenyldiazomethane was added to the filtrate.

The mixture was left to stand for 2 hours and concentrated under reduced pressure. To the residue was added 500 mℓ. of ether, followed by stirring. Produced precipitates were collected on a filter to give 12 g. of diphenylmethyl 7-phenoxyacetamido-3-hydroxymethyl-3-cephem-4-carboxylate.

NMR (deutro DMSO) δ ppm

3.65 (2H, s, CH_2 at 2-position)
4.29 (2H, d, J = 3 Hz, CH_2 at 3-position)
4.65 (2H, s, CH_2)
5.17 (1H, t, J = 3 Hz, OH at 3-position)
5.19 (1H, d, J = 2.5 Hz, 6-position)
5.76 (1H, d · d, J = 2.5, 4.5 Hz, 7-position)
6.7 - 7.8 (16H, m)
9.11 (1H, d, J = 4.5 Hz, NH)

In 400 mℓ. of dry methylene chloride was dissolved 5 g. of diphenylmethyl 7-phenoxyacetamido-3-hydroxymethyl-3-cephem-4-carboxylate. 0.1 mℓ. of boron trifluoride ethyl etherate was added to the above solution. Excess diazomethane (about 3 g., an ether solution prepared from 21.4 g. of N-methyl-N-nitroso-p-toluenesulfonamide was heated and produced gas was bubbled) was reacted with the solution, followed by stirring for a day. The reaction mixture was concentrated under reduced pressure and the residue was purified by chromatography through 250 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (3 : 2) to

afford 4.3 g. of diphenylmethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCl_3$) δ ppm

3.20 (3H, s, OCH_3 at 3-position)

3.50 (2H, s, CH_2 at 2-position)

4.25 (2H, s, CH_2 at 3-position)

4.57 (2H, s, CH_2)

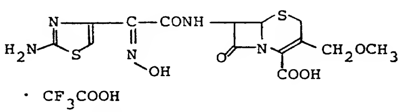
5.00 (1H, d, J = 2.5 Hz, 6-position)

5.87 (1H, d, d, J = 2.5, 4.5 Hz, 7-position)

6.7 - 7.6 (17H, m)

Referential Example 4

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid trifluoroacetic acid salt



168 mg. of diketene was dissolved in 2 mℓ. of methylene chloride, followed by cooling at -30 °C with stirring. To the solution was added dropwise a solution of 320 mg. of bromine in 2 mℓ. of methylene chloride. The resulting solution was added dropwise to 5 mℓ. of a methylene chloride solution containing 362 mg. of diphenylmethyl 7-amino-3-methoxymethyl -3-cephem-4-carboxylate and 299 mg. of diethylaniline,

which had been cooled to -5 °C. The mixture was left to stand for 30 minutes to complete the reaction. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in 50 mℓ. of ethyl acetate, washed successively with each 5 mℓ. of water, a 5% aqueous hydrochloric acid solution and a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated. The residue thus obtained was chromatographed through 30 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to afford 118 mg. of diphenylmethyl bromoacetylacetamido-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 5 mℓ. of acetic acid. To the solution was added little by little 16 mg. of sodium nitrite with stirring at room temperature, followed by stirring for 30 minutes. The reaction mixture was diluted with 20 mℓ. of ethyl acetate, washed three times with a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the solution was concentrated under reduced pressure. The residue thus obtained was chromatographed through 10 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to give 76 mg. of diphenylmethyl 7-(2-bromoacetyl-2-hydroxyiminoacetamido)-3-methoxymethyl-3-cephem-4-carboxylate. 76 mg. of the compound obtained above was dissolved in 3 mℓ. of N,N-dimethylacetamide and 19 mg.

of thiourea was added thereto, followed by stirring for 2 hours. The reaction mixture was added to 20 mℓ. of ethyl acetate. The mixture was washed enough with a saturated aqueous sodium bicarbonate solution to remove the excess thiourea and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue thus obtained was chromatographed through 5 g. of silica gel eluted with ethyl acetate to give 49 mg. of diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. 49 mg. of the compound thus obtained was dissolved in 1 mℓ. of anisole and 0.5 mℓ. of trifluoroacetic acid was added thereto. The mixture was left to stand at room temperature for an hour. The reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in 1 mℓ. of acetone and 20 mℓ. of isopropyl ether was added thereto. Produced precipitates were collected on a filter and dried to afford 28 mg. of 7-[2-(2-aminothiazol-4-)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid trifluoroacetic acid salt.

NMR (deutromethanol d_4) δ ppm

3.28 (3H, s, OCH_3 at 3-position)

3.55 (2H, s, CH_2 at 2-position)

4.29 (2H, s, CH_2 at 3-position)

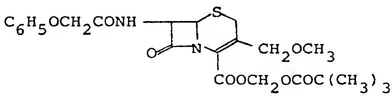
5.11 (1H, d, J = 2.5 Hz, 6-position)

5.81 (1H, d, J = 2.5 Hz, 7-position)

6.83 (1H, s)

Referential Example 5

Preparation of Pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate



In 50 mℓ. of dimethyl sulfoxide was dissolved 1 g. of sodium 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate and 975 mg. of pivaloyloxymethyl bromide was added thereto, followed by stirring at room temperature for 15 minutes. The reaction mixture was diluted with 200 mℓ. of ethyl acetate, washed successively with 50 mℓ. of a saturated aqueous sodium bicarbonate solution and 50 mℓ. of a saturated aqueous potassium bisulfate solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue thus obtained was chromatographed through 100 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to afford 750 mg. of pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCℓ_3$) δ ppm

1.25 (9H, s)

3.35 (3H, s, OCH_3 at 3-position)

3.54 (2H, s, CH_2 at 2-position)

4.29 (2H, s, CH_2 at 3-position)

4.58 (2H, s, $\underline{CH_2}$-O-Phe)

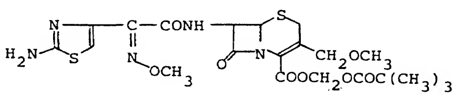
5.01 (1H, d, J = 2.5 Hz, 6-position)

5.6 - 6.1 (3H, m, 7-position and CH_2)

6.7 - 7.6 (6H, m, Phenyl and NH)

Example 1

Preparation of Pivaloyloxymethyl 7-[2-(2-aminothiazol -4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate



In 5 mℓ. of dry methylene chloride was dissolved 488 mg. of phosphorus pentachloride and 120 mg. of phosphorus oxychloride was added thereto. Under stirring at room temperature, 247 mg of pyridine was added to the above mixture. The mixture was cooled to -10 °C and 769 mg. of pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate was added thereto. The temperature of the mixture was elevated to room temperature step by step. After stirring for 2 hours, the reaction mixture was cooled to 0 °C again. 1.5 mℓ. of n-propyl alcohol was added to the

mixture, followed by stirring for 30 minutes. A small amount of water was added to the mixture, followed by stirring for 15 minutes. The mixture was diluted with 50 mℓ. of ethyl acetate and washed with a saturated aqueous sodium bicarbonate solution. The ethyl acetate layer was dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. Isopropyl ether was added to the residue and the wall of the vessel was rubbed. Produced precipitates were collected on a filter and dried to give 443 mg. of pivaloyloxymethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate. According to Referential Example 1, 121 mg. of pivaloyloxymethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate thus obtained was subjected to amidoation, using 141 mg. of diethylaniline, 71 mg. of dimethylformamide, 135 mg. of phosphorus oxychloride and 265 mg. of 2-(2-chloroacetamidothiazol -4-yl)-2-methoxyiminoacetic acid. The reaction mixture was extracted and the extract was concentrated. The resulting residue was chromatographed trough 10 g. of silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (2 : 1) to afford 55 mg. of pivaloyloxymethyl 7β-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 1 mℓ. of N,N-dimethylacetamide and 13.5 mg. of thiourea was added thereto, followed by stirring at room temperature for 2 hours. The

reaction mixture was diluted with 20 mℓ. of ethyl acetate, washed with a saturated aqueous sodium bicarbonate solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed through 5 g. of silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (3 : 1) to afford 36 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR (deutroacetone) δ ppm

1.19 (9H, s)

3.23 (3H, s, OCH_3 at 3-position)

3.52 (2H, s, CH_2 at 2-position)

3.90 (3H, s, OCH_3)

4.18 (2H, s, CH_2 at 3-position)

5.12 (1H, d, J = 2.5 Hz, 6-position)

5.8 - 6.1 (3H, m, 7-position and CH_2)

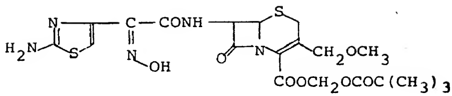
6.78 (1H, s)

6.6 - 7.1 (2H, bs, NH_2)

8.01 (1H, d, J = 4.5 Hz, NH)

Example 2

Preparation of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate



According to Referential Example 4, 168 mg. of diketene, 320 mg. of bromine, 322 mg. of pivaloyloxymethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate and 299 mg. of diethylaniline were subjected to the reaction and the resulting crude product was purified by chromatography through 30 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to give 288 mg. of pivaloyloxymethyl 7-bromoacetylacetamido-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 5 mℓ. of acetic acid and treated with 38 mg. of sodium nitrite following the same procedure as in Referential Example 4. The resulting crude product was chromatographed through 15 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to afford 200 mg. of pivaloyloxymethyl 7-(2-bromoacetyl-2-hydroxyiminoacetamido)-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 5 mℓ. of N,N-dimethylacetamide and treated with 55 mg. of thiourea following the same procedure as in Referential Example 4. The product thus obtained was purified by chromatography through 10 g. of silica gel eluted with ethyl acetate to give 118 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR (deutroacetone) δ ppm

1.21 (9H, s)

3.28 (3H, s, OCH_3 at 3-position)

3.61 (2H, s, CH_2 at 2-position)

4.27 (2H, s, CH_2 at 3-position)

5.21 (1H, d, J = 2.5 Hz, 6-position)

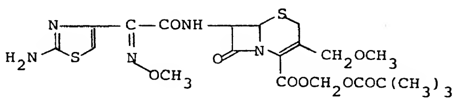
5.8 - 6.2 (3H, m, 7-position and CH_2)

6.87 (1H, s)

6.4 - 7.6 (3H, m, NH_2, OH)

9.0 (1H, d, J = 4.5 Hz, NH)

Example 3

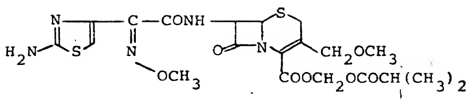


To 10 mℓ. of dimethyl sulfoxide were added 1 g. of 7-[2-(2-chloroacetamidothiazol -4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid (syn isomer), 380 mg. of bromomethyl pivalate and 240 mg. of potassium fluoride, followed by stirring at room temperature for an hour. The reaction mixture was diluted with 100 mℓ. of ethyl acetate and washed successively with water, a 5% aqueous sodium bicarbonate solution, a 10% aqueous potassium bisulfate solution and a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The

solvent was distilled off under reduced pressure. The residue was chromatographed through silica gel eluted with a mixed solvent of chloroform and ethyl acetate (1 : 1) to give 300 mg. of pivaloyloxymethyl 7-[2-(2-chloroacetamido-thiazole-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate as a pale yellow powder.

The compound obtained above and 60 mg. of thiourea were dissolved in 3 mℓ. of dimethylacetamide, followed by stirring at room temperature for 4 hours. The reaction mixture was poured into 10 mℓ. of a saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The extract was successively washed with a 10% aqueous potassium bisulfate solution and a saturated aqueous sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography through silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (3 : 1) to give 200 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl) -2-methoxyimino-acetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The compound obtained above was identified to be the some compound as that obtained in Example 1 by comparing its unclear magnetic resonance spectrum and infrared spectrum with those of the compound obtained in Example 1.

Example 4



Following the procedure of Example 3, but replacing bromomethyl pivalate with 360 mg. of bromomethyl isobutyrate, there was obtained 180 mg. of isobutyryloxymethyl 7-[2-(2-aminothiazol -4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate as a slightly yellow powder.

NMR ($CDC\ell_3$) δ ppm:

1.20 (6H, d, J = 6.5)

2.66 (1H, septet, J = 6.5)

3.21 (3H, s)

3.40 (2H, ABq)

4.01 (3H, s)

4.16 (2H, s)

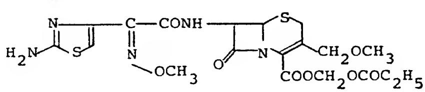
5.05 (1H, d, J = 5)

5.6 - 6.2 (5H, m)

6.65 (1H, s)

8.06 (1H, d, J = 9)

Example 5



Following the procedure of Example 3, but replacing bromomethyl pivalate with 340 mg. of bromomethyl propionate, there was obtained 165 mg. of propionyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate as an almost colourless powder.

NMR ($CDCl_3$) δ ppm:

1.17 (3H, t, J = 6.5)

2.41 (2H, q, J = 6.5)

3.20 (3H, s)

3.35 (2H, ABq)

4.02 (3H, s)

4.17 (2H, s)

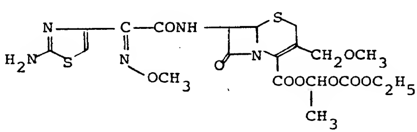
5.09 (1H, d, J = 5)

5.6 - 6.3 (5H, m)

6.68 (1H, s)

8.25 (1H, d, J = 9)

Example 6



Following the procedure of Example 3, but replacing bromomethyl pivalate with 600 mg. of α-ethoxycarbonyloxyethyl bromide (prepared by heating 600 mg of α-ethoxycarbonyloxy-

ethyl chloride and 800 mg. of sodium bromide in 3 mℓ. of acetonitrile for 10 hours), there was obtained 60 mg. of 1-ethoxycarbonyloxyethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate as a pale yellow powder.

NMR ($CDC\ell_3$) δ ppm

1.30 (3H, t, J = 7)

1.61 (3H, d, J = 5)

3.22 (3H, s)

3.42 (2H, ABq)

4.03 (3H, s)

4.15 (2H, s)

4.21 (2H, q, J = 7)

5.10 (1H, d, J = 5)

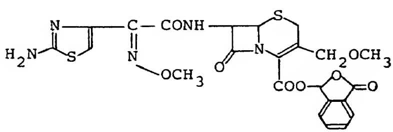
5.6 - 6.2 (3H, m)

6.70 (1H, s)

6.92 (1H, q)

8.20 (1H, d, J = 9)

Example 7



To 10 mℓ. of dimethyl sulfoxide were added, in turn, 1 g. of 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid (syn isomer), 200 mg. of triethylamine and 420 mg. of phthalidyl bromide. The mixture was stirred at room temperature for 30 minutes, diluted with 100 mℓ. of ethyl acetate, washed successively with water, a 5% aqueous sodium bicarbonate solution, a saturated aqueous potassium bisulfate solution and a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure. The resulting residue was purified by column chromatography through silica gel eluted with a mixed solvent of chloroform and ethyl acetate (1 : 1) to afford 710 mg. of phthalidyl 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate as a pale yellow powder.

300 mg. of the above compound was reacted with 60 mg. of thiourea in 3 mℓ. of dimethylacetamide, and the mixture was left to stand at room temperature for 4 hours to complete the reaction. The reaction mixture was treated according to the procedure of Example 3 to obtain 120 mg. of phthalidyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCℓ_3$) δ ppm:

3.22 (3H, s)

3.50 (2H, s)

3.95 (3H, s)

4.17 (2H, s)

5.10 (1H, d)

5.82 (1H, m)

6.58 (0.6H, s)

6.67 (0.4H, s)

7.3 - 8.3 (5H, m)

8.5 (2H, br.)

Example 8

According to the procedure of Example 2, there are obtained following compounds.

(1) Acetoxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

(2) Isovaleryloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

(3) Phthalidyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

Example 9

According to the procedure of Example 1, there are obtained following compounds.

(1) Acetoxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

(2) Isovaleryloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

(3) Pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-ethoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate

(4) Pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-ethoxymethyl-3-cephem-4-carboxylate

Example 10

2 mℓ. of an ether solution saturated with hydrogen chloride was added to a solution of 500 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate in 20 mℓ. of ethyl acetate. The reaction mixture was concentrated under reduced pressure to a volume of about 5 mℓ. and 20 mℓ. of diisopropyl ether was added thereto. Produced precipitates were collected on a filter, washed with diisopropyl ether and dried to afford 480 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate hydrochloride. Yield, 480 mg.

According to the above procedure, 500 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate was treated

to give 470 mg. of its hydrochloride.

Patent Applicant	Sankyo Co., Ltd.
Agent Patent Attorney	Shoji Kashiide

Written Supplement of Proceedings (Voluntary)

November 11, 1980

To : Haruki Shimada, Director-General of the Patent Office

1. Indication of the case

Patent Application No. 136449/1980

2. Title of the invention

Cephalosporin compounds for oral administration

3. Person making the supplement

Relation with the case Patent Applicant

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Name Patent Attorney (6007) Shoji Kashiide

Seal

5. Number of invention to be increased by supplement: None

6. Subject matter to be supplemented

Detailed explanation of the invention in the specification

7. Contents of supplement

As given in the annexed paper

1. Specification page 16, lines 3 and 2 from the bottom; insert "N-hydroxybenztriazole" after "N-hydroxyphthalimide ester".
2. Ditto, page 29, line 6; amend "J = 2.5 Hz" to read "J = 5 Hz".
3. Ditto, page 29, line 7; amend "J = 2.5, 4.5 Hz" to read "J = 5, 9 Hz".
4. Ditto, page 31, lines 12 and 13; amend "J = 2.5 Hz" to read "J = 5 Hz".
5. Ditto, page 33, lines 3 and 5; amend "J = 3 Hz" to read "J = 6 Hz".
6. Ditto, page 33, line 6; amend "J = 2.5 Hz" to read "J = 5 Hz".
7. Ditto, page 33, line 7; amend "J = 2.5, 4.5 Hz" to read "J = 5, 9 Hz".
8. Ditto, page 33, line 9; amend "J = 4.5 Hz" to read "J = 9 Hz".
9. Ditto, page 34, line 10; amend "J = 2.5 Hz" to read "J =5 Hz".
10. Ditto, page 34, line 11; amend "J = 2.5, 4.5 Hz" to read "J = 5, 9 Hz".
11. Ditto, page 38, lines 1 and 2; amend "J = 2.5 Hz" to read "J = 5 Hz".
12. Ditto, page 39, line 14; amend "J = 2.5 Hz" to read "J = 5 Hz".

13. Ditto, page 42, line 3 from the bottom; amend "J = 2.5 Hz" to read "J = 5 Hz".

14. Ditto, page 43, line 2; amend "J = 4.5 Hz" to read "J = 9 Hz".

15. Ditto, page 45, line 3; amend "J = 2.5 Hz" to read "J = 5 Hz".

16. Ditto, page 45, line 7; amend "J = 4.5 Hz" to read "J = 9 Hz".

Written Supplement of Proceedings (Voluntary)

July 16, 1981

To : Haruki Shimada, Director-General of the Patent Office

1. Indication of the case

Patent Application No. 136449/1980

2. Title of the invention

Cephalosporin compounds for oral administration

3. Person making the supplement

Relation with the case Patent Applicant

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Name Patent Attorney (6007) Shoji Kashiide

Seal

5. Number of invention to be decreased by supplement: 1

6. Subject matter to be supplemented

Detailed explanation of the invention in the specification

7. Contents of supplement

As given in the annexed paper

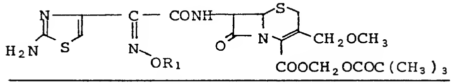
AMENDED SPECIFICATION

1. Title of the Invention

Cephalosporin compounds for oral administration

2. Scope of Patent Claim

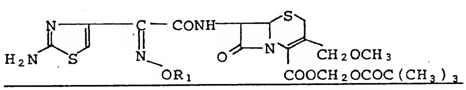
(1) A cephalosporin compound (syn isomer) having the general formula



(wherein, R_1 represents methyl or ethyl group)

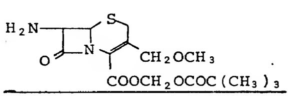
and the pharmacologically acceptable acid addition salt thereof.

(2) A process for preparing a cephalosporin compound (syn isomer) having the general formula

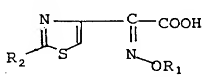


(wherein, R_1 represents methyl or ethyl group)

or the pharmacologically acceptable acid addition salt thereof, which comprises steps of reacting a compound having the general formula

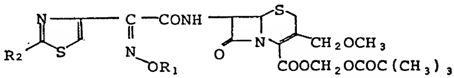


with a carboxylic acid having the general formula



(wherein, R_2 represents amino group or a protected amino group, and R_1 is as defined above)

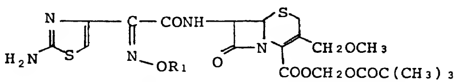
to give a compound having the general formula



(wherein, R_1 and R_2 are as defined above),

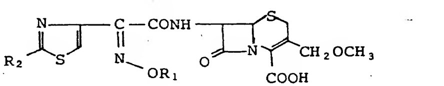
and, when R_2 is a protected amino group, removing the protecting group from the resulting compound.

(3) A process for preparing a cephalosporin compound (syn isomer) having the general formula



(wherein, R_1 is methyl or ethyl group)

or the pharmacologically acceptable acid addition salt thereof, which comprises steps of reacting a carboxylic acid compound having the general formula



(wherein, R_2 is amino group or a protected amino group; and, R_1 is as defined above)

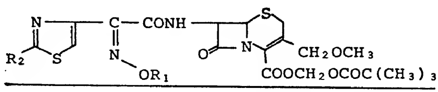
or a reactive derivative thereof with a compound having the

general formula

$$XCH_2OCOC(CH_3)_3$$

(wherein, X represents a halogen atom)

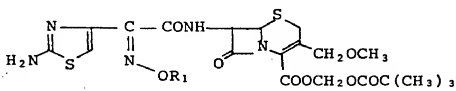
to give a compound having the general formula



(wherein, R_1 and R_2 are as defined above),

and, when R_2 is a protected amino group, removing the protecting group from the resulting compound.

(4) An oral treating agent for infectious disease comprising a cephalosporin compound (syn isomer) having the general formula



(wherein, R_1 represents methyl or ethyl group)

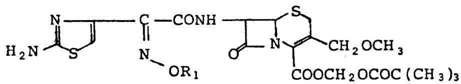
or the pharmacologically acceptable acid addition salt thereof.

2. Detailed Description of the Invention

This invention relates to cephalosporin compounds for oral administration.

More particularly, it relates to cephalosporin compounds

(*syn* isomer) having the general formula



(wherein, R_1 represents methyl or ethyl group) and the pharmacologically acceptable acid addition salts thereof, processes for preparing said compounds and oral treating agents for infectious diseases comprising said compounds as the active ingredient.

The present compounds having the above formula (I) are novel compounds which are readily absorbed through the digestive tract and converted *in vivo* to a carboxylic acid type compounds by elimination of the ester moiety at the 4-position. Thus, it is possible to obtain a high concentration of the carboxylic acid type compound in blood and to achieve a highly remarkable effect in treatment of infectious diseases caused by gram-positive and gram-negative bacteria when administered orally. The carboxylic acid type compounds are also novel and have excellent antibacterial activities as described later.

Although there are many penicillin and cephalosporin type antibiotics which exhibit excellent effects, very rarely seen are the compounds which are absorbed efficiently through the digestive tract. For this reason, there are a number of compounds of which development has been given up. Among the

antibiotics of cephalosporin type, only compounds having a limited structure such as cephalexin or its analogs are offered for practical purposes.

The recovery ratio in urine of cephalosporin preparations widely used for injections such as cephalothin, cefazolin, cefmethazole, when administered orally, is about 5% of the dose administered, and they are known for their extremely poor absorption through the digestive tract. The reason for this is because dissociation of carboxylic group at 4-position of cephalosporin is great (pKa value is small) and the acidity is strong.

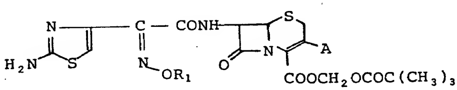
Efforts have been made to improve such absorption through the digestive tract by esterifying carboxylic groups at 3-position of penicillins or at 4-position of cephalosporins. There are one or two cases where such efforts have matured into commercialization in respect of penicillin compounds. But so far none succeeded for all efforts in the case of cephalosporin compounds.

There is a report in the Journal of Antibiotics, Vol. 32, No. 11, p. 1155 (1979) regarding acetoxymethyl ester of cefamandol. According to this report, the absorption is not improved because the esterification makes the compound only slightly soluble in water, and the absorption is improved to a certain degree only when it is administered in the solution of an organic solvent such as propylene glycol. On

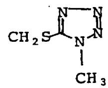
the other hand, there is a report on an ester which is easily soluble in water in the Journal of Medicinal Chemistry, Vol. 22, p. 657 (1979) which further describes that absorption was not improved because the compound was chemically unstable.

The inventors of the present invention have conducted extensive research for many years on the improvement of absorption of cephalosporin compounds through digestive tracts by chemical modifications and on elevation of concentration in blood through oral administrations. The inventor has learned that these properties are related to the whole structure of the compounds, and the absorption through the digestive tract is utterly unpredictable even when the similar chemical modification such as esterification is conducted so long as a part of the structure is different. The present invention was achieved based on the result of such researches.

The inventor synthesized pivaloyloxymethyl esters of the known compound identified below which have seemingly similar structure as the compounds of the present invention, and conducted experiments to study the recovery ratio thereof in urine through oral administration. The results obtained were not necessarily good, and the inventor found that the substituent at 3-position plays an extremely significant role as far as the compounds of this group are concerned.



Compound 1 $R_1 = CH_3$ $A = CH_2OCOCH_3$

Compound 2 $R_1 = CH_3$ $A =$ 

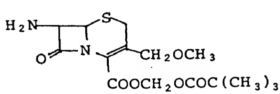
The present compounds having the formula (I) can be prepared by the following methods:

(a) Acylation of the esterified 7-amino-3-methoxymethyl-cephalosporin (II).

(b) Esterification of the carboxylic acid (I) corresponding to the compound (I) or its amino-protected derivative (V) and elimination of the protecting groups.

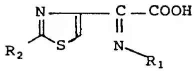
The above methods will more concretely be illustrated below.

(a) The compound (I) is prepared by reacting a compound having the general formula



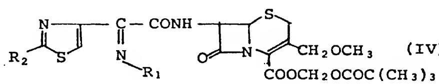
(II)

with a carboxylic acid having the general formula



(III)

(wherein R_2 represents amino group or a protected amino group and R_1 represents methyl or ethyl group) or its reactive derivative to give a compound having the general formula



(IV)

(wherein R_1 and R_2 are as defined above) and, when R_2 is the protected amino group, removing the protecting group.

In the above formulae, preferred amino-protecting groups in R_2 are those which are readily removed to restore amino group, for example, trityl group, formyl group, t-butoxycarbonyl group, or 2-ethoxycarbonyl-1-methylvinyl group, which are removable by acid treatment, 2-methylsulfonylethyloxycarbonyl group, which is removable by alkali treatment, and chloracetyl group, which is removable by treatment with thiourea.

The present process consists of two steps, namely an acylation step and, if necessary, a removing step of the protecting group.

In the acylation step, the compound having the formula (III) is used in a free form or in a form of the reactive derivative. When it is used as such, a suitable condensing agent is employed. Examples of such condensing agent include a di-substitued carbodiimide such as dicyclohexylcarbodiimide, an imidazolide such as carbonyldiimidazole or thionyldiimidazole, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, or a Vilsmeier reagent prepared from dimethylformamide and, e.g., phosphorus oxychloride or thionyl chloride. Examples of the reactive derivative of the compound having the formula (III) include the acid halide, the acid anhydride, the mixed acid anhydride, the reactive ester, the reactive amide, the acid azide, etc. Preferred mixed acid anhydrides include the mixed acid anhydride with carbonic acid mono lower alkyl ester such as monomethyl carbonate or monoisobutyl carbonate or the mixed acid anhydride with a lower alkanoic acid such as pivalic acid or trichloroacetic acid. Preferred reactive esters include p-nitrophenyl ester, pentachlorophenyl ester, N-hydroxyphthalimide ester, N-hydroxybenztriazole ester, etc.

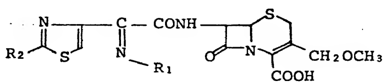
The present step is preferably conducted in a solvent. The solvent is not limited as far as it does not obstruct the reaction and exemplified by an inert organic solvent such as acetone, methyl ethyl ketone, tetrahydrofuran, dioxane, ethyl acetate, chloroform, dichloromethane,

acetonitrile,dimethylformamide, dimethyl sulfoxide, etc. or a mixture of said solvent and water. Depending on a kind of the reactive derivative employed, a base is used, if necessary. Examples of the base include an alkali metal compound, for example, sodium bicarbonate, potassium bicarbonate, sodium carbonate, potassium carbonate, etc. and an aliphatic, aromatic or nitrogen-containing heterocyclic base, for example, triethylamine, dimethylaniline, diethylaniline, N-methylpiperidine, N-methylpyrrolidine, pyridine, collidine, lutidine, etc. The reaction temperature is not limited and usually the reaction is conducted at room temperature or under cooling. The reaction period may be varied depending on mainly acylation methods and reaction temperature, but usually from tens of minutes to tens of hours. After completion of the reaction, the compound having the formula (IV) may be recovered from the reaction mixture by conventional means. For example, where water-miscible solvent is employed, the solvent is removed by distillation under reduced pressure and the residue is dissolved in a water-immiscible solvent. The resulting solution is washed with an acid and base and dried and the solvent is distilled off to obtain the product. Where water-immiscible solvent is employed, the reaction mixture is directly washed with an acid and base. The product thus obtained, if necessary, is further purified by conventional means, for example,

chromatographic techniques.

In the removing step of the protecting group, the protecting group may be removed, as mentioned above, by conventional means according to the properties thereof and the resulting crude product is purified to give the desired compound having the formula (I).

(b) Alternatively, the compound (I) can be prepared by reacting the carboxylic acid compound having the general formula



(V)

(wherein R_1 and R_2 are as defined above)

or its reactive derivative with a compound having the general formula

$$XCH_2OCOC(CH_3)_3 \qquad (VI)$$

(wherein X represents a halogen atom such as chlorine, bromine or iodine)

to give the compound having the above formula (IV) and, when R_2 is a protected group, removing the protecting group from the resulting compound as mentioned in the method (a).

In the above reaction, the compound (V) may be used in the form of a reactive derivative advantageous for the

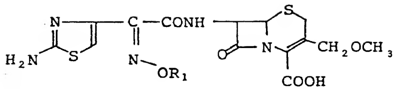
condensation. Examples of the reactive derivatives of the carboxyl moiety in the compound (V) include the salt with a metal such as sodium or potassium, the salt with an organic amine such as triethylamine or dicyclohexylamine. The reaction is desirably conducted in the presence of a suitable solvent which does not obstruct the reaction. Examples of such solvent include dimethylformamide, dimethylacetamide, dimethyl sulfoxide, hexamethyltriamidophosphate, acetonitrile, etc. or the mixture with other inert organic solvent. The reaction is usually conducted at room temperature or under cooling. The reaction period is usually from several minutes to several hours. After completion of the reaction, the reaction mixture is diluted with a water-immiscible solvent, washed successively with an aqueous potassium bisulfate solution and an aqueous basic solution and dried and the solvent is distilled off to obtain the desired product. The product thus obtained may be further purified by conventional means, for example, chromatographic techniques. Where the amino group is protected, the group is converted to free amino group, following the procedure applied in the above compound (IV).

The compounds (I) thus obtained are, as mentioned above, readily absorbed through the digestive tract and give a high concentration of the carboxylic acid type compound in blood and, accordingly, oral administration of the compound is

possible. The compound can be formulated with conventional pharmaceutical vehicles to form capsules, powders, granules, tablets. The pharmaceutical vehicles include diluents, for example, starch, lactose, sugar, calcium carbonate, calcium phosphate, polyethylene glycol etc., binding agents, for example, gum arabic, carboxymethyl cellulose, hydroxypropyl cellulose, etc., lubricants, for example, magnesium stearate, talc, sodium laurylsulfate, etc. and disintegrants, for example, carboxymethyl cellulose calcium salt, etc. The dose will vary depending upon age, body weight and condition of patient. From about 0.2 g. to 5 g., preferably from 0.5 g. to 3 g. of the compound can be administered daily to the human adult in 3 or 4 devided doses.

The compounds having the formula (I) can be used, as well as in a form of a free base, in a form of pharmacologically acceptable acid addition salts, for example, the salt with an inorganic acid such as hydrochloric acid, sulfuric acid or phosphoric acid, or the salt with an organic acid such as methanesulfonic acid, benzenesulfonic acid, malonic acid, etc.

The present compounds (I), as mentioned above, are readily absorbed through the intestine by oral administration and hydrolyzed *in vivo* to produce the corresponding carboxylic acid (I') or the salt thereof.

 (I')

(wherein R_1 is as defined above) .

Antibacterial activities of the compound (I') against gram-positive and gram-negative bacteria (minimal inhibitory concentrations μg/mℓ) are extraordinarily potent as shown below.

	Compound (I') A	Compound (I') B
	$R_1=CH_3$	$R_1=C_2H_5$
Staphylococcus aureus 209 P	0.4	0.2
Escherichia coli NIHJ	0.4	0.8
Shigella flexneri	0.8	0.4
Klebsiella pneumoniae	0.1	0.2
Proteus vulgaris	0.01	0.01
Salmonella enteritidis	0.2	0.4

The present compounds and the related compounds mentioned herein above (Compound 1 and Compound 2) were applied orally to mice and their recovery rates in urine (i.e., the amounts of the corresponding carboxylic acids) are given below.

	Recovery rate in urine (%, 0∿24 hours)
Compound in Example 1	75.9
Compound in Example 2	78
Compound 1	8
Compound 2	14

As shown above, the present invention is characterized by the improvement in absorbability of cephalosporin compounds through the digestive tract by converting the substituents of the Compound 1 and Compound 2 at 3-position to methoxymethyl group.

The processes for preparing the present compounds having the above-mentioned formula (I) will be concretely illustrated by the following Referential Examples and Examples, which are not to be construed as limiting the scope of the invention. The present compounds take syn form with respect to the oxime moiety.

Referential Example 1.

Pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate

In 50 mℓ. of dimethyl sulfoxide was dissolved 1 g. of sodium 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate and 975 mg. of pivaloyloxymethyl bromide was

added thereto, followed by stirring at room temperature for 15 minutes. The reaction mixture was diluted with 200 mℓ. of ethyl acetate, washed successively with 50 mℓ. of a saturated aqueous sodium bicarbonate solution and 50 mℓ. of a saturated aqueous potassium bisulfate solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue thus obtained was chromatographed through 100 g. of silica gel eluted with a mixed solvent of n-hexane and ethyl acetate (1 : 1) to afford 750 mg. of pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCℓ_3$) δ ppm

1.25 (9H, s)

3.35 (3H, s, OCH_3 at 3-position)

3.54 (2H, s, CH_2 at 2-position)

4.29 (2H, s, CH_2 at 3-position)

4.58 (2H, s, CH_2-O-Phe)

5.01 (1H, d, J=2.5 Hz, 6-position)

5.6 - 6.1 (3H, m, 7-position and CH)

6.7 - 7.6 (6H, m, Phenyl and NH)

Referential Example 2.

(a) <u>Diphenylmethyl 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate</u>

(Reaction solution (A))

To 0.057 mℓ. of dimethylformamide was added 0.061 mℓ. of phosphorus oxychloride under ice-cooling and stirring. The mixture was stirred at 40 °C for an hour and subjected twice to azeotropy with dry methylene chloride. To the resulting mixture was added 1 mℓ. of ethyl acetate. Under vigorously stirring at room temperature, 200 mg. of 2-(2-chloroacetamidothiazole-4-yl)-2-methoxyiminoacetic acid was added to the above mixture and stirring was continued for 30 minutes.

(Mixing solution (B))

On the other hand, 200 mg. of diphenylmethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate and 145 mg. of diethylaniline were dissolved in 5 mℓ. of methylene chloride and stirred at -5 °C.

To the resulting mixture (B) was added dropwise the above reaction mixture (A). After stirring for 15 minutes, the reaction mixture was concentrated under reduced pressure. To the residue were added 20 mℓ. of ethyl acetate and 5 mℓ. of water. The ethyl acetate layer was separated, washed successively with 5 mℓ. of a saturated aqueous sodium bicarbonate solution, 5 mℓ. of a 5% hydrochloric acid and 5 mℓ. of a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under

reduced pressure. The residue was chromatographed through 30 g. of silica gel (Kieselgel-60) eluted with a mixed solvent of n-hexane and ethyl acetate (3 : 2) to give 213 mg. of diphenylmethyl 7-[2-(2-chloroacetylaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR ($CDCl_3$) δ ppm

3.19 (3H, s, OCH_3 at 3-position)

3.51 (2H, s, CH_2 at 2-position)

4.09 (3H, s, OCH_3)

4.20 (2H, s, CH_2 at 3-position)

4.22 (2H, s, $ClCH_2 \cdot CO$)

5.02 (1H, d, J=2.5 Hz, 6-position)

5.86 (1H, d.d, J=2.5, 4.5 Hz, 7-position)

6.7 - 7.6 (12H, m)

(b) <u>7-[2-(2-Chloroacetaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid</u>

7.65 g. of diphenylmethyl 7-[2-(2-chloroacetaminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate were reacted with 25 mℓ. of methylene dichloride, 5 mℓ. of anisole and 20 mℓ. of trifluoroacetic acid, by a usual method, at room temperature for 30 minutes. After completion of the reaction, 300 mℓ. of isopropyl ether were added, and the precipitates were collected by filtration to give 5.95 g. of 7-[2-(2-chloroacetamidothiazol-4-yl)-2-

methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid (syn isomer).

NMR (Mixture of deuteroacetone and deuterodimethyl sulfoxide) δ ppm

3.30 (3H, s, OCH_3 at 3-position)

3.60 (2H, s, CH_2 at 2-position)

3.97 (3H, s, OCH_3)

4.25 (2H, s, $-CH_2-$ at 3-position)

4.37 (2H, s, $C\ell CH_2CO$)

5.20 (1H, d, H at 6-position)

5.90 (1H, d.d, H at 7-position)

7.40 (1H, s, 5-position of thiazole)

9.50 (1H, d, CONH at 7-position)

Referential Example 3.

7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate trifluoroacetate

In 5 mℓ. of N,N-dimethylacetamide were successively dissolved 200 mg. of diphenylmethyl 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyimonoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate and 45 mg. of thiourea. The solution was maintained at room temperature for 2 hours. After addition of a saturated aqueous sodium bicarbonate solution, the reaction mixture was extracted with 20 mℓ. of ethyl acetate. The ethyl acetate layer was washed with water to remove the excess thiourea and dried over anhydrous magnesium sulfate.

After the drying agent was filtered off, the filtrate was concentrated under reduced pressure. The resulting residue was chromatographed through 30 g. of silica gel (Wacogel C-100) eluted with ethyl acetate to afford 63 mg. of diphenylmethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 2 mℓ. of anisole and 1 mℓ. of trifluoroacetic acid was added thereto under ice-cooling and stirring. The mixture was maintained at room temperature for 30 minutes. The reaction mixture was concentrated under reduced pressured and isopropyl ether was added thereto. Produced precipitates were collected on a filter and dried to afford 27 mg. of 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid trifluoroacetic acid salt.

NMR (in deuteroacetone, heavy water was added): δ ppm

3.29 (3H, s, $-OCH_3$ at 3-position)

3.57 (2H, s, CH_2 at 2-position)

3.96 (3H, s, OCH_3)

4.27 (2H, s, CH_2 at 3-position)

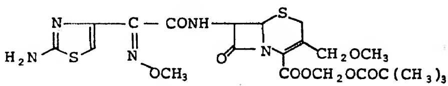
5.15 (1H, d, J=2.5 Hz, 6-position)

5.97 (1H, d, J=2.5 Hz, 7-position)

6.59 (1H, s)

Example 1

Pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyimino-acetamido]-3-methoxymethyl-3-cephem-4-carboxylate



In 5 mℓ. of dry methylene chloride was dissolved 488 mg. of phosphorus pentachloride and 120 mg. of phosphorus oxychloride was added thereto. Under stirring at room temperature, 247 mg. of pyridine was added to the above mixture. The mixture was cooled to -10 °C and 769 mg. of pivaloyloxymethyl 7-phenoxyacetamido-3-methoxymethyl-3-cephem-4-carboxylate was added thereto. The temperature of the mixture was elevated to room temperature step by step. After stirring for 2 hours, the reaction mixture was cooled to 0 °C again. 1.5 mℓ. of n-propyl alcohol was added to the mixture, followed by stirring for 30 minutes. A small amount of water was added to the mixture, followed by stirring for 15 minutes. The mixture was diluted with 50 mℓ. of ethyl acetate and washed with a saturated aqueous sodium bicarbonate solution. The ethyl acetate layer was dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. Isopropyl ether was added to the residue and the wall of the vessel was scraped. Produced precipitates were collected on a filter and

dried to give 443 mg. of pivaloyloxymethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate. According to Referential Example 2-(a), 121 mg. of pivaloyloxymethyl 7-amino-3-methoxymethyl-3-cephem-4-carboxylate thus obtained were subjected to amidoation, using 141 mg. of diethylaniline, 71 mg. of dimethylformamide, 135 mg. of phosphorus oxychloride and 265 mg. of 2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetic acid. The reaction mixture was extracted and the extract was concentrated. The resulting residue was chromatographed through 10 g. of silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (2 : 1) to afford 55 mg. of pivaloyloxymethyl 7β-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The product was dissolved in 1 mℓ. of N,N-dimethylacetamide and 13.5 mg. of thiourea was added thereto, followed by stirring at room temperature for 2 hours. The reaction mixture was diluted with 20 mℓ. of ethyl acetate, washed with a saturated aqueous sodium bicarbonate solution and dried over anhydrous magnesium sulfate. The drying agent was filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed through 5 g. of silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (3 : 1) to afford 36 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate.

NMR (deuteroacetone) δ ppm

1.19 (9H, s)

3.23 (3H, s, OCH_3 at 3-position)

3.52 (2H, s, CH_2 at 2-position)

3.90 (3H, s, OCH_3)

4.18 (2H, s, CH_2 at 3-position)

5.12 (1H, d, J=2.5 Hz, 6-position)

5.8 - 6.1 (3H, m, 7-position and CH_2)

6.78 (1H, s)

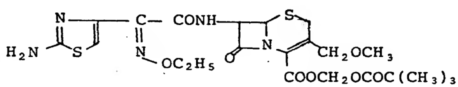
6.6 - 7.1 (2H, bs, NH_2)

8.01 (1H, d, J=4.5 Hz, NH)

Example 2

Following the method of Example 1, the following compound was obtained.

Pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-ethoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate



NMR (deuterochloroform) δ ppm

1.22 (9H, s)

1.31 (3H, t, OCH_2CH_3)

3.30 (3H, s, OCH_3)

3.53 (2H, s, CH_2 at 2-position)

4.30 (2H, s, CH_2 at 3-position)

4.28 (2H, q, OCH_2CH_3)

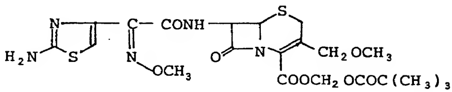
5.01 (1H, d, J=5 Hz, 6-position)

5.7 – 6.2 (5H, m, H at 7-position, NH_2, $COOCH_2$–O–)

6.76 (1H, s, 5-position of thiazole)

7.70 (1H, d, J=9 Hz, CONH)

Example 3



To 10 mℓ. of dimethyl sulfoxide were added 1 g. of 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylic acid (syn isomer) obtained in Referential Example 2-(b), 380 mg. of bromomethyl pivalate and 240 mg. of potassium fluoride, followed by stirring at room temperature for an hour. The reaction mixture was diluted with 100 mℓ. of ethyl acetate and washed successively with water, a 5% aqueous sodium bicarbonate solution, a 10% aqueous potassium bisulfate solution and a saturated aqueous sodium chloride solution and dried over anhydrous magnesium sulfate. The solvent was distilled off under reduced pressure. The residue was chromatographed through silica gel eluted with a mixed solvent of chloroform and ethyl acetate (1 : 1) to give 300 mg. of pivaloyloxymethyl 7-[2-(2-chloroacetamidothiazol-4-yl)-2-methoxylimino-acetamido]-3-methoxymethyl-3-cephem-4-carboxylate as a pale

yellow powder.

The compound obtained above and 60 mg. of thiourea were dissolved in 3 mℓ. of dimethylacetamide, followed by stirring at room temperature for 4 hours. The reaction mixture was poured into 10 mℓ. of a saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The extract was successively washed with a 10% aqueous potassium bisulfate solution and a saturated aqueous sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography through silica gel eluted with a mixed solvent of ethyl acetate and n-hexane (3 : 1) to give 200 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate. The compound obtained above was identified to be the same compound as that obtained in Example 1 by comparing its nuclear magnetic resonance spectrum and infrared spectrum with those of the compound obtained in Example 1.

Example 4

2 mℓ. of an ether solution saturated with hydrogen chloride was added to a solution of 500 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate in 20 mℓ. of ethyl acetate. The reaction mixture was concentrated under reduced pressure to a volume of about 5 mℓ. and 20 mℓ. of

diisopropyl ether was added thereto. Produced precipitates were collected on a filter, washed with diisopropyl ether and dried to afford 470 mg. of pivaloyloxymethyl 7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methoxymethyl-3-cephem-4-carboxylate hydrochloride.

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